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Comparative Enzyme Induction and Lindane Metabolism in Rats Pre-treated with Various Organochlorine Pesticides

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16. ABSTRACT

1. The comparative effect of 7 days pre-treatment with one of seven organochlorine pesticides on the metabolism of lindane *in vivo* and on the metabolism of EPN, *p*-nitroanisole and methyl orange *in vitro* was investigated.

2. Mirex was the most potent inducer of the oxidative hydrolysis of EPN, the *O*-demethylation of *p*-nitroanisole and the azo reduction of methyl orange. Chlordane and DDT were equipotent followed by hexachlorobenzene, toxaphene, pentac and penphene. Enzyme activity of rats pre-treated with the latter three pesticides was not significantly higher than that of controls.

3. DDT stimulated significantly greater metabolism of lindane than did pre-treatment with any of the other six pesticides. In general DDT, mirex, chlordane and hexachlorobenzene were the most effective activators of lindane metabolism followed by toxaphene, pentac and penphene. Only pre-treatment with penphene failed to stimulate a significantly higher excretion of radiolabelled lindane metabolites than that excreted by controls.

4. Comparative differences in lindane metabolism *in vivo* were not adequately reflected by the determination of enzyme activities *in vitro*.

5. Pre-treatment with organochlorine pesticides altered lindane metabolism by selective effects on specific metabolic pathways.

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Comparative Enzyme Induction and Lindane Metabolism in Rats Pre-treated with Various Organochlorine Pesticides

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5. Pre-treatment with organochlorine pesticides altered lindane metabolism by selective effects on specific metabolic pathways.

Introduction

Enzyme induction is currently regarded as an adaptive response regulating the detoxication of foreign compounds, although many examples have been cited of the metabolism of xenobiotics to chemically reactive and toxic metabolites (Gillette, Mitchell & Brodie, 1974). Stimulation of drug metabolism by organochlorine pesticides has been extensively studied by many investigators who have concluded that the mechanism involves induction of liver microsomal enzymes (Street, 1968). Based primarily on data from comparative enzyme assays *in vitro*, barbiturates, miscellaneous drugs and organochlorine pesticides are believed to bring about a non-specific induction of microsomal enzymes through similar mechanisms (Mullen, Juchau & Fouts, 1966). However, qualitative differences in the stimulation of selected enzyme activities by various drugs and pesticides have been reported (Lange, 1967; Kato, Takayanagi & Oshima, 1969) and results from other studies have indicated that the metabolism of toxicants *in vivo* may be selectively altered by pre-treatment with different pesticides and drugs (Koransky *et al.*, 1964; Street & Chadwick, 1967; Street, 1968; Chadwick & Freal, 1972 a). Furthermore, Gillette (1971) has cautioned

that differences in the metabolism of foreign compounds *in vivo* may be due to factors that are difficult, if not impossible, to evaluate with tissue homogenates.

This study examines the comparative effect of seven days' pre-treatment with one of seven pharmacologically related but structurally dissimilar organochlorine pesticides on the metabolism of lindane *in vivo* and on the activity of various rat liver microsomal enzymes *in vitro*.

Experimental

Chemicals

All chemicals other than those noted below were obtained from commercial sources.

All pesticides were obtained from the Pesticides and Toxic Substance Effects Laboratory Analytical Reference Standards Repository, Research Triangle Park, N. C. The purities of DDT, hexachlorobenzene (HCB), mirex, penphene and pentac were 99·9%, 98%, 98%, 99·1% and 98% respectively. Chlordane and toxaphene were technical grade and contained 100% of the active ingredient. The lindane metabolite 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol (PCCOL) was isolated from urine as described previously (Chadwick & Freal, 1972 b).

Animals, dosing, and specimen collection

Forty-eight weanling female Holtzman rats, housed in individual metabolism cages, were randomly assigned to one of eight treatment groups. These groups received daily oral injections of solutions of arachis oil containing 1 mg of either chlordane, DDT, hexachlorobenzene (HCB), mirex, penphene, pentac, toxaphene or arachis oil alone (Fig. 1). On the eighth day, all animals received 1·7 mg of lindane (containing 1·40 μCi of [^{14}C]lindane) in place of their previous treatment. The [^{14}C]lindane (obtained from Amersham/Searle, Arlington Heights, Ill., U.S.A.) was uniformly labelled, had a specific activity of 48 Ci/mol, and a radiochemical purity of 99%. Twenty-four hours later, the rats were sacrificed by decapitation to avoid any complications due to anaesthetics. Urine, faeces, fat, liver and kidney samples were taken for analysis of radioactivity. Liver samples were homogenised and the activity *in vitro* of various microsomal enzymes was determined. Twenty-four hour urine samples were analysed by g.l.c. for known metabolites of lindane (Chadwick & Freal, 1972 b).

Radioactivity counting

Aliquots of urine and extracts of urine, liver and fat samples were analysed for radioactivity with a Nuclear Chicago scintillation counter (Mark I) using a scintillation solution containing 500 ml toluene, 500 ml cellosolve, 8·0 g of 2,5-diphenyloxazole (PPO) and 50 mg of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP). Dried samples of faeces and kidney were analysed for radioactivity after combustion by a method developed by Watts, Locke and Baron (1969) in which $^{14}CO_2$ is trapped in a solution containing 55% v/v toluene, 20% v/v methanol, 20% v/v 2-phenylethylamine, 5% v/v triton X-100, 0·55% w/v PPO and 0·01% w/v POPOP.

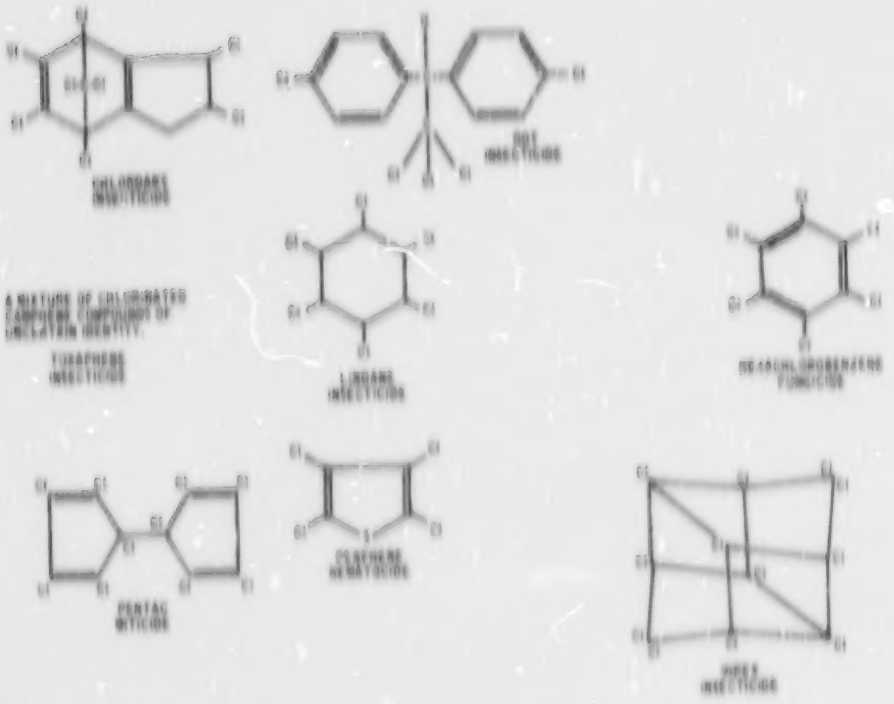

Fig. 1. *The organochlorine pesticides employed in this study.*

Enzyme assays

A 1 g portion of each liver was homogenized with 4 ml of 1·15% KCl in a Potter–Elvehjem homogenizer with a motor-driven Teflon pestle. The homogenization was carried out at 4° for 1 min. The enzyme activity involved in the *O*-demethylation of *p*-nitroanisole (Kinoshita, Frawley & DuBois, 1966), the oxidative hydrolysis of *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioic acid (EPN) (Kinoshita *et al.*, 1966) and the reduction of the azo group of sodium *p*-dimethylaminoazobenzenesulphonate (methyl orange) (Fouts, Kamm & Brodie, 1957) was determined.

Chromatography

Urines were collected and extracted as described elsewhere (Chadwick *et al.*, 1975 a). Metabolites were analysed on a Tracor Model MT 220 gas chromatograph equipped with an electron capture detector and a Coulson electrolytic conductivity detector, operated in the oxidative mode. The column used consisted of a 183 × 0·63 cm o.d. glass U-tube packed with 5% DEGS (Analabe Inc. Hamden, Conn., U.S.A. on 80–100 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa., U.S.A.) isothermally maintained at 180°. The flow of nitrogen carrier gas was regulated a 90 cm^3/min.

Statistics

Duncan's Multiple Range Test was used as an aid in the interpretation of the data from this study (Duncan, 1955). Comparisons were considered significantly different at $P < 0.05$ unless otherwise stated.

Results

Liver and body weight

Pre-treatment with either mirex or penphene significantly depressed body weight while pre-treatment with hexachlorobenzene (HCB) increased body weight (Table 1). On the other hand, rats receiving mirex had significantly larger livers than all other animals in the study. Chlordane, DDT and hexachlorobenzene-treated rats also had significantly larger livers than control animals.

Table 1. Effect of pre-treatment of rats on body weight, liver weight, and hepatic microsomal enzyme activity

Pre-treatment	Body wt. (g)	Liver wt. (g)	Substrate metabolised: EPN*	Substrate metabolised: *p*-Nitroanisole*	Substrate metabolised: Methyl orange†
1. Control	133 ± 3·23	5·80 ± 0·26	3·67 ± 0·28	4·40 ± 0·24	9·5 ± 1·74
2. Chlordane	123 ± 3·88	9·15 ± 0·24	7·85 ± 1·11	8·35 ± 0·83	15·5 ± 3·62
3. DDT	130 ± 3·64	7·93 ± 0·35	7·15 ± 0·53	8·83 ± 0·38	19·0 ± 4·82
4. HCB	139 ± 3·24	7·50 ± 0·33	4·58 ± 0·47	7·33 ± 0·63	13·2 ± 2·02
5. Mirex	117 ± 6·10	9·54 ± 0·52	11·00 ± 1·17	9·97 ± 0·90	24·8 ± 1·04
6. Penphene	116 ± 4·12	6·60 ± 0·42	5·15 ± 0·32	5·33 ± 0·26	11·8 ± 1·95
7. Pentac	123 ± 4·73	6·10 ± 0·32	3·43 ± 0·44	5·13 ± 0·48	10·5 ± 0·84
8. Toxaphene	126 ± 4·41	6·34 ± 0·44	4·50 ± 0·17	6·08 ± 0·36	13·7 ± 1·91
Treatment Comparison‡	1,1 > 5,6 4 > 2,7	5 > All 3,3,4 > 7,1 2,3 > 6,8	5 > All 3,3 > 4,8,7,6,1	5,3,2,4 > 6,7,1 5,3,2 > 8 5 > 4	5,3,2 > 6,7,1 5 > 8,4

Values are means ± SE. Pre-treatments are described in methods.

* Activity expressed as μg of *p*-nitrophenol liberated per 30 mg of liver per hour using the whole liver homogenate.

† Activity expressed as μg of methyl orange reduced per 30 mg of liver per 30 min using the whole liver homogenate.

‡ Comparisons reported are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

Enzyme activity

Liver homogenates from rats pre-treated with mirex showed significantly higher activity in the oxidative hydrolysis of EPN than all other animals. These rats also had significantly greater *O*-demethylase and azo reductase activity than all animals except those treated with DDT or chlordane (Table 1). Rats pre-treated with chlordane or DDT had higher enzyme activity than the remaining treatment groups for metabolism of EPN and higher *O*-demethylase

than all rats except those receiving mirex or HCB. Hexachlorobenzene pre-treatment resulted in significantly greater O-demethylase activity than pre-treatment with pentac or penphene. Finally, pre-treatment with DDT or chlordane induced significantly higher azo reductase than pre-treatment with pentac or penphene. Pre-treatment with toxaphene, penphene or pentac did not significantly increase enzyme activity during the period of this study.

Excretion of radioactivity after administration of [^{14}C]lindane

For all animals most of the excreted radioactivity was found in the urine. Radioactivity in the faeces was between 2–3% of the total excreted (Table 2). Only penphene pre-treatment failed to stimulate a significantly higher excretion of radioactivity than that from the controls. In general DDT, mirex, chlordane and hexachlorobenzene stimulated lindane metabolism to a greater extent than toxaphene, pentac and penphene. Rats pre-treated with DDT excreted significantly more radioactivity, with 64·1% of the administered dose, than all other animals. This contrasts with the fact that mirex produced the greatest *in vitro* enzyme activity.

Based on the proportion of radioactivity found in the urinary extracts, the effect of pre-treatment on the distribution of lindane metabolites was determined (Table 3). While DDT and HCB pre-treated rats excreted almost 40% of their lindane metabolites as neutrals and free chlorophenols, the animals exposed to chlordane or mirex excreted only 20% of the metabolites as neutrals and free chlorophenols. On the other hand, the reverse was true for the proportions of conjugated chlorophenols and polar metabolites excreted. Chlordane- and mirex- pre-treated rats excreted almost 80% whereas DDT- and HCB-treated rats excreted about 60% as conjugated chlorophenols and polar metabolites.

Lindane storage

Rats pre-treated with the organochlorine pesticides that stimulated the greatest metabolism, stored the least lindane in their tissue (Table 2). There was a highly significant negative correlation ($r = -0{\cdot}88$) between total excreted radioactivity and lindane stored in fat lipid for all treatment groups.

Excretion of PCCOL

The effect of pre-treatment on the metabolism of lindane to 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol (PCCOL) is presented in Table 4. Both the amount excreted per 24 h and the percentage of the neutral metabolites which this represents are given. Rats pre-treated with HCB excreted a significantly higher proportion of this neutral metabolite than the controls, or rats pre-treated with chlordane, mirex or penphene. Pre-treatment with pentac, DDT and toxaphene also promoted the excretion of a significantly greater percentage of this metabolite than penphene pre-treatment. Mirex and chlorane stimulated this pathway much less than would have been expected from their comparative potency as enzyme inducers *in vitro* (Table 1).

Total chlorophenol excretion

While the DDT-pretreated rats excreted significantly more total chlorophenols than the other animals, they excreted significantly less conjugated

Table 2. Storage and excretion of ^{14}C after treatment of rats with [^{14}C]fenchone

Pre-treatment	Total excreted radioactivity		Radioactivity in urine		Radioactivity in faeces		Stored fenchone		
	dpm × 10^{-6}	%Adm. dose	dpm × 10^{-6}	%Adm. dose	dpm × 10^{-6}	%Adm. dose	Kidney*	Liver*	Fat†
1. Control	5·14 ± 0·50	12·7%	5·02 ± 0·50	12·4%	0·12 ± 0·03	0·30%	6·91 ± 0·79	9·57 ± 1·60	3170 ± 690
2. Chlordane	19·5 ± 1·04	48·4%	19·0 ± 1·05	47·1%	0·45 ± 0·07	1·17%	5·20 ± 0·86	4·78 ± 0·31	5715 ± 339
3. DDT	25·9 ± 0·73	64·2%	25·1 ± 0·69	62·2%	0·86 ± 0·08	1·98%	2·64 ± 0·36	2·78 ± 0·13	3644 ± 147
4. HCB	14·1 ± 1·79	35·0%	13·7 ± 1·75	34·0%	0·37 ± 0·05	0·92%	5·94 ± 1·44	5·26 ± 0·52	6817 ± 940
5. Mirex	21·7 ± 1·68	53·8%	21·2 ± 1·70	52·6%	0·47 ± 0·09	1·16%	6·12 ± 0·91	4·84 ± 0·82	5912 ± 735
6. Perphene	6·58 ± 0·61	16·3%	6·41 ± 0·61	15·9%	0·17 ± 0·06	0·42%	6·89 ± 1·22	7·98 ± 0·36	12864 ± 778
7. Photox	9·88 ± 0·96	24·5%	9·70 ± 0·98	24·0%	0·18 ± 0·03	0·45%	7·56 ± 1·62	7·50 ± 1·79	9717 ± 1514
8. Toxaphene	13·1 ± 0·98	32·0%	13·0 ± 0·99	32·2%	0·30 ± 0·02	0·74%	6·20 ± 0·41	6·85 ± 0·35	7219 ± 370
Treatment Comparisons‡	3>All 5,2>4,8,7,6,1 4,8>7,6,1 7>6,1		3>All 5,2>4,8,7,6,1 4,8>7,6,1 7>6,1		3>All 5,2,4>6,1 5,2>7		4,7,1,8,5,4>3 6>2	1,7,6,8>3 1,7>2 8,4,5<1	All>3 6,1,7,8>5 6,1,7>8,4,2 6>7

Values are means ± SE.

Pre-treatments are described in Methods.

* p.p.m. fenchone based on ^{14}C present in tissue.

† p.p.m. fenchone based on ^{14}C present in tissue lipid.

‡ Comparisons reported are significantly different at $P < 0.05$ by Duncan's Multiple Range Test.

Table 5. Effect of pre-treatment of rats on the proportions of radiolabelled lindane metabolites excreted in urine

Pre-treatment	Average percentage of radioactivity excreted in urine extracts			
	Neutral metabolites*	Free chlorophenols†	Conjugated chlorophenols‡	Polar metabolites§
1. Control	4·43 ± 1·34	25·5 ± 1·43	26·0 ± 2·32	45·4 ± 3·54
2. Chlordane	1·82 ± 0·34	19·9 ± 3·20	38·0 ± 4·24	40·0 ± 4·20
3. DDT	4·48 ± 1·06	38·0 ± 1·74	27·0 ± 1·71	33·6 ± 3·01
4. HCB	3·18 ± 0·36	35·0 ± 4·33	27·6 ± 3·32	34·2 ± 6·45
5. Mires	1·64 ± 0·25	19·1 ± 3·25	33·4 ± 1·14	46·0 ± 3·36
6. Penphene	2·50 ± 0·26	21·6 ± 2·13	26·2 ± 1·45	49·8 ± 3·43
7. Pentac	3·77 ± 0·62	25·6 ± 1·84	28·2 ± 2·57	42·4 ± 1·78
8. Toxaphene	4·18 ± 1·74	29·1 ± 4·82	30·6 ± 1·71	36·2 ± 6·49
Treatment	N.S.	3,4 > 1,6,2,5 > 8 > 5	2 > 7,4,3,1,6	6 > 4,5
Comparisons¶		3,4 > 6,2,5 > 8 > 5		2,5,6 > 4,5

Values are means ± SE. Pre-treatments are described in Methods.

* % Radioactivity extracted with benzene from acid urine (pH = 2) which does not partition into 0·1 M NaOH.

† % Radioactivity extracted with benzene from acid urine (pH = 2) which does partition into 0·1 M NaOH.

‡ % Radioactivity extracted with benzene from hydrolysed urine.

§ % Radioactivity remaining in residual urine after extraction.

¶ Comparisons reported are significantly different at $P = 0·05$ by Duncan's Multiple Range Test.

chlorophenols (33%) than either the mires- (57%) or chlordane-treated (61%) rats (Table 4). This could be explained if the sulphotransferase and/or glucuronyltransferase systems had been maximally activated and/or if the conjugation were limited by the availability of a key component such as the co-substrate adenosine-3'-phosphate-5'-phosphosulphate (PAPS). However work in this laboratory with rats fed diets containing 350 p.p.m. lindane for 1 week has indicated that from a total chlorophenol excretion greater than 800 μg/24 h, more than 80% was conjugated (unpublished data). Thus it appeared that chlordane and mires promote conjugation to a greater extent than DDT.

Proportion of individual chlorophenols excreted

The effect of pre-treatment with the seven organochlorine pesticides on the relative proportion of excreted chlorophenol metabolites is presented in (Table 5, Fig. 3). The excretion patterns consist of the relative proportions of each of the five lindane metabolites: 2,4,6-, 2,3,5-, 2,4,5-trichlorophenol, 2,3,4,6- and 2,3,4,5-tetrachlorophenol. Pre-treatment with the organochlorine pesticides used in this study significantly altered the chlorophenol excretion pattern when compared with that of the control animals. For example there was a marked

Table 4. Effect of pre-treatment on the proportions of 2,3,4,5,6-pentachloro-2-cyclohexene-1-ol (PCCHL), free and conjugated chlorophenols excreted in urine after treatment of rats with lindane

Pre-treatment	PCCHL*		Total free chlorophenols*		Total conjugated chlorophenols*	
	μg Excreted/24 h	% PCCHL in neutral extract	μgExcreted/24 h	% of Total chlorophenols	μg Excreted/24 h	% Total of chlorophenols
1. Control	1·30 ± 0·38	14·0 ± 2·32	46·5 ± 4·04	65·2 ± 6·45	26·7 ± 6·45	34·8 ± 6·45
2. Chlordane	2·36 ± 0·56	15·1 ± 1·69	131 ± 28·1	38·5 ± 7·66	212 ± 47·7	61·5 ± 7·66
3. DDT	9·60 ± 3·20	38·7 ± 1·76	506 ± 29·4	66·5 ± 5·16	244 ± 90	33·4 ± 5·1a
4. HCB	4·60 ± 0·68	29·4 ± 2·61	169 ± 35·4	56·8 ± 7·60	152 ± 49·0	43·2 ± 7·60
5. Mirex	2·15 ± 0·42	14·7 ± 1·85	146 ± 17·1	42·7 ± 6·40	236 ± 77·5	57·3 ± 6·40
6. Propheno	0·80 ± 0·27	10·3 ± 2·65	41·8 ± 4·49	55·6 ± 6·94	38·9 ± 13·0	44·4 ± 6·94
7. Fenitc	3·02 ± 0·92	29·4 ± 2·88	89·4 ± 4·36	54·8 ± 6·67	89·5 ± 20·5	45·2 ± 6·67
8. Toxaphene	7·65 ± 5·51	18·8 ± 4·52	144 ± 39·0	47·4 ± 9·67	155 ± 37·5	52·6 ± 9·68
Treatment Comparisons†	3>2,5,1,6	4>2,5,1,6 7,3,8>6	3>4,8 4,5,8,2>1,6 4>7	3,1>2 3>5	1,5,2,>6,3 3>7	2,5>3 2>1

Values are means ± SE. Pre-treatments are described in Methods.

* Quantities of excreted metabolites were determined by g.l.c. analysis.

† Comparisons reported are significantly different at $P < 0{\cdot}05$ by Duncan's Multiple Range Test.

Table 5. Effect of pre-treatment of rats on the proportions of the individually excreted chlorophenols after treatment with Endosan

Pre-treatment	Average percentage of total chlorophenol excretion					Ratio*
	2,4,6-TCP	2,3,5-TCP	2,4,5-TCP	2,3,4,6-TTCP	2,3,4,5-TTCP	
1. Control	46·4 ± 1·13	7·45 ± 0·59	12·3 ± 1·21	17·7 ± 1·38	16·0 ± 1·96	2·71 ± 0·25
2. Chlordane	42·2 ± 1·25	8·40 ± 0·23	9·95 ± 0·90	33·8 ± 1·58	5·56 ± 0·94	1·27 ± 0·05
3. DDT	25·6 ± 0·78	5·10 ± 0·22	10·7 ± 0·28	34·8 ± 1·31	23·8 ± 1·36	0·74 ± 0·02
4. HCB	37·4 ± 1·60	7·52 ± 0·41	12·4 ± 0·85	37·3 ± 1·46	9·42 ± 1·02	1·04 ± 0·05
5. Mirex	44·2 ± 0·94	10·9 ± 0·64	5·69 ± 0·66	24·7 ± 0·55	5·41 ± 1·06	1·28 ± 0·06
6. Pentphene	42·5 ± 1·27	9·78 ± 0·85	10·2 ± 0·99	22·3 ± 1·06	16·2 ± 3·05	1·95 ± 0·33
7. Pentac	40·4 ± 1·33	8·10 ± 0·70	12·4 ± 1·04	20·6 ± 1·95	9·36 ± 1·90	1·37 ± 0·08
8. Tetraphene	39·7 ± 1·50	6·72 ± 0·22	11·8 ± 0·80	15·2 ± 0·80	7·46 ± 1·03	1·10 ± 0·06
Treatment	All>3	All>3	All>5	All>1	3>All	1>All
	1,5,6,2>4	5,6,2>8		8,3,5,2,4,7>6	6,1>4,7,8,2,5	6>7,5,2,4,8
Comparisons†	1,5>8	5>2,7,4,1		8,3,5>7		
	1>4,2,7					

Values are means ± SE. Pre-treatments are described in Methods.

Abbreviations:

2,4,6-TCP, 2,4,6-trichlorophenol
2,3,5-TCP, 2,3,5-trichlorophenol
2,4,5-TCP, 2,4,5-trichlorophenol
2,3,4,6-TTCP, 2,3,4,6-tetrachlorophenol
2,3,4,5-TTCP, 2,3,4,5-tetrachlorophenol

* μg 2,4,6-TCP/μg 2,3,4,6-TTCP

† Comparisons reported are significantly different at $P < 0{\cdot}05$ by Duncan's Multiple Range Test.

RELATIVE CHLORPHENOL EXCRETION
(% DOSE)
CONJUGATED
FREE
RELATIVE CHLORPHENOL EXCRETION
(% DOSE)

decline in the ratio of 2,4,6-trichlorophenol to 2,3,4,6-tetrachlorophenol excreted by treated rats. Pre-treatment with even penphene, which had negligible effects on other responses (Tables 1–4), significantly decreased this ratio. This suggests that the 2,4,6-trichlorophenol to 2,3,4,6-tetrachlorophenol ratio may serve as a sensitive index of toxicant exposure. Furthermore the excretion patterns may be viewed as a kind of fingerprint, useful in the characterisation of toxicant exposure. For example, DDT exposure resulted in the excretion of a significantly greater proportion of 2,3,4,5-tetrachlorophenol than the other treatment groups while mirex pre-treatment significantly decreased the proportion of 2,4,5-trichlorophenol excreted and rats receiving chlordane excreted a significantly greater proportion of conjugated 2,3,4,6-tetrachlorophenol than all animals but those treated with mirex and toxaphene.

Discussion

Results from this study indicated that there were significant and rather selective changes in lindane metabolism in rats pre-treated with chlordane, DDT, HCB, mirex, penphene, pentac or toxaphene (Tables 2–5, Fig. 2). Though the first step in the metabolism of lindane involves a cytochrome P-450-dependent mixed-function oxidase (Chadwick, Chuang & Williams, 1975 b), the comparative differences in lindane metabolism *in vivo* were not adequately reflected by routine enzyme assays involving liver homogenates. For example, while mirex pre-treatment induced the highest enzyme activity *in vitro* (Table 1), DDT pre-treatment stimulated the greatest lindane metabolism *in vivo* (Table 2). Moreover, whereas chlordane and DDT appeared to be equipotent in stimulating enzyme activity *in vitro*, DDT was much superior in promoting lindane metabolism. While neither pentac nor toxaphene pre-treatment induced significantly higher enzyme activity *in vitro* than the controls, both pentac and toxaphene significantly increased the excretion of radiolabelled lindane metabolites (Table 2). Furthermore in contrast to their effects on enzyme activity *in vitro*, neither mirex nor chlordane stimulated the lindane to PCCOL pathway (Table 4).

In addition to the uniform increase in lindane metabolism, which one might expect from pre-treatment of rats with similarly acting enzyme inducers of different potency, there were quantitative differences in the excretion of certain specific metabolites. The pre-treatment influenced the overall proportions of the various lindane metabolites excreted (Table 3), the conjugation of the chlorophenols formed (Table 4), and the excretion pattern of the individual chlorophenol metabolites (Table 5, Fig. 2) and PCCOL (Table 4). Each pre-treatment alters some pathways more than others and is characteristic of the toxicant employed. This selectivity may even be employed as a 'fingerprint' of toxicant exposure. This implies that assays *in vitro*, involving specific enzymes, are not completely satisfactory for evaluating the rather selective effects of toxicants on the metabolism of xenobiotics when multiple pathways are involved.

Microsomal enzyme systems *in vitro* are routinely employed to study the extent and rate of drug metabolism and what effect factors such as enzyme stimulators, enzyme inhibitors and various disease states may have on this metabolic activity (Gillette, 1971; Remmer, 1972; Conkeley & Powell, 1971; Rumack, Holtsman & Chase, 1973; Rosen *et al.*, 1971). However, Gillette

(1971) warned that differences in the metabolism of foreign compounds in vivo may not be adequately evaluated with tissue homogenates. Results from this study support this assertion and indicate that some enzyme inducers, currently believed to act through a similar mechanism, may actually alter toxicant metabolism by rather selective effects on specific metabolic pathways.

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